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ORIGINAL PAPER

A consensus linkage map identifies genomic regions controlling fruit maturity and beta-carotene-associated flesh color in melon (*Cucumis melo* L.)

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Abstract The nutritional value and yield potential of US Western Shipping melon (USWS; *Cucumis melo* L.) could be improved through the introgression of genes for early fruit maturity (FM) and the enhancement of the quantity of β-carotene (QβC) in fruit mesocarp (i.e., flesh color). Therefore, a set of 116 F_3 families derived from the monoecious, early FM Chinese line 'Q 3-2-2' (no β-carotene, white mesocarp) and the andromonoecious, late FM USWS line 'Top Mark' (possessing β-carotene, orange mesocarp) were examined during 2 years in Wisconsin, USA to identify quantitative trait loci (QTL) associated with FM and QβC. A 171-point F_{2-3} based map was constructed and used for QTL analysis. Three QTL associated with QβC were detected, which explained a significant portion of the observed phenotypic variation (flesh color; $R^2 = 4.0$–50.0%). The map position of one QTL (β-carM.E.9.1) was uniformly aligned with one carotenoid-related gene (Orange gene), suggesting its likely role in QβC in this melon population and putative relationship with the melon white flesh (wf) gene. Two major (FM.6.1 and FM.11.1; $R^2 \geq 20\%$) and one minor QTL (FM.2.1; $R^2 = 8\%$) were found to be associated with FM. This map was then merged with a previous recombinant inbred line (RIL)-based map used to identify seven QTL associated with QβC in melon fruit. This consensus map [300 molecular markers (187 co-dominant melon and 14 interspecific; 10 LG)] provides a framework for the further dissection and cloning of published QTL, which will consequently lead to more effective trait introgression in melon.

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Introduction

Carotenoids are a class of compounds produced by most photosynthetic organisms that are essential to both plants and animals (Wong et al. 2004). They play essential functions in plants including roles in phyto-hormone precursor action (Schwartz et al. 2003) and environmental adaptation through modulation of the photosynthetic apparatus (Demming-Adams and Adams 2002). Moreover, some carotenoid pigments (e.g., α-carotene and β-carotene) are important for human health and nutrition due to their function in vitamin A biosynthesis (Mares-Perlman et al. 2002), and strong evidence suggests that diets rich in carotenoids (e.g., β-carotene, lycopene) can prevent the onset of some chronic diseases (Mares-Perlman et al. 2002) and certain cancers (e.g., prostate cancer) (Giovannucci 2002).

Melon (*Cucumis melo* L.; $2n = 2x = 24$) is an economically important, cross-pollinated vegetable species that is grown worldwide. The nutritional value of melon is variable, and dependent upon the market class, genotype and growing environment (Lester and Eischen 1995). Fruit of orange-fleshed (mesocarp) market types [e.g., US Western Shipping (USWS); Group Cantalupensis] are a rich source of dietary carotenes (primarily β-carotene) (Kläui and

Bauernfeind 1981; Gross 1987), and are therefore an important source of vitamin A.

Fruit maturity [i.e., number of days from sowing to first mature fruit; FM (IPGRI 2003)] is an important trait in plant breeding (Hancoq et al. 2004), and its inheritance in melon is complex and highly affected by the environment (Zalapa et al. 2006, Monforte et al. 2004). Early fruit maturation can improve crop marketability by increasing harvest number (i.e., more production cycles per year). Heritability of days to anthesis is moderate (0.57–0.62) with a significant proportion of its variance being additive (Zalapa et al. 2006) and correlated to early fruit maturation ($r = 0.24$, Zalapa et al. 2007) in USWS market types. However, germplasm with early FM and enhanced quantity of β-carotene (QβC) content is not publicly available.

Genes for green (gf; Hughes 1948) and white (wf; Imam et al. 1972) flesh color have been previously reported, but the genetics involved in melon mesocarp color has not been clearly defined. Clayberg (1992) indicated that green and white flesh are recessive to orange, where gf and wf interact epistatically, such that wf^+_/gf^+_ and wf^+_/gfgf genotypes produce fruit with an orange mesocarp, the genotype wfwf/gf^+_ bears fruits having a white mesocarp, and the wfwf/gfgf genotype develops fruit that possess a green mesocarp. However, this genetic model was not confirmed in subsequent mapping populations developed from exotic PI accessions and elite varieties differing in fruit mesocarp color (Perin et al. 2002; Monforte et al. 2004; Fukino et al. 2008). Although the analysis of populations in succeeding studies confirmed the recessive inheritance of green flesh color, the inheritance of orange fruit mesocarp color has remained ambiguous. For instance, Fukino et al. (2008) and Perin et al. (2002) described simple inheritance (1:1) of mesocarp color in recombinant inbred lines (RIL) derived from the cross 'AR 5' (orange-fleshed) × 'Harukei 3' (green-fleshed) and PI 161375 (green mesocarp) × 'Véndrantais' (orange mesocarp), respectively. These studies placed white (wf) and green flesh (gf) gene (Fukino et al. 2008; Perin et al. 2002, respectively) on the same linkage group (LG IX), but a later analysis (Monforte et al. 2004) indicated that the gf mapped by Perin et al. (2002) was, in fact, wf. Monforte et al. (2004) also mapped gf to the distal region of LG 1 and characterized three quantitative trait loci (QTL; ofc2.1, ofc3.1, and ofc12.1) associated with orange flesh color. However, these QTL were not confirmed by comparative analysis of nearly isogenic lines derived from the same mapping population (Eduardo et al. 2007). More recently, Cuevas et al. (2008) identified QTL associated with QβC using a population of RIL derived from two parental lines ('USDA 846-1' and 'Top Mark') that possessed orange flesh color. That study identified and mapped seven QTL, where four (β-car.1.1, β-car.2.1, β-car.4.2 and β-car.6.2) and two (β-car.2.2 and β-car.6.1) QTL were location specific for California and Wisconsin, respectively, and one QTL (β-car.4.1) was location independent. Moreover, the map locations of two QTL (β-car.6.2 and β-car.6.1) were colinear with a previously reported fruit color QTL (ofc12.1; Monforte et al. 2004).

The recent dramatic increase in the number of published simple sequence repeat (SSR) melon markers (>400; Chiba et al. 2003; Ritchel et al. 2004; Kong et al. 2007; Gonzalo et al. 2005; Fukino et al. 2007; Fernandez-Silva et al. 2008) and genomics resources [e.g., expressed sequence tag (EST) libraries; cucurbit genomics resource (http://cucurbit.bti.cornell.edu)] affords opportunities for map merging to increase map saturation in melon and for colinearity analyses. Given the need for more saturated melon maps and the economic importance of FM and QβC for melon improvement, an SSR-based map was constructed using an F_{2-3} family analysis to: (1) identify QTL associated with FM and QβC (flesh color); (2) merge this map with a previous RIL-based map with attending QTL associated with QβC (Cuevas et al. 2008), yield (Zalapa et al. 2007) and fruit quality components (Paris et al. 2008); and (3) map putative carotenoid biosynthetic genes, which may allow for candidate gene analysis using QTL related to flesh color and QβC in melon.

Materials and methods

Plant materials

The monoecious, early flowering and fruiting (maturity) Chinese melon line 'Q 3-2-2' (P_1) that develops fruit with a white mesocarp (i.e., white flesh) was crossed to the andromonoecious, comparatively late flowering (typically 21 days after P_1) and late fruiting breeding line 'Top Mark' (P_2) that produces fruit with an orange mesocarp (i.e., orange flesh). While Group Cantalupensis 'Top Mark' belongs to the USWS market class and has been used in previous mapping population (Cuevas et al. 2008), line 'Q 3-2-2' does not fall into any common market class and has genetic affinities to Group Conomon and Momordica melons (Luan et al. 2008). A single F_1 plant from this initial mating was self-pollinated to produce F_2 individuals. A total of 116 F_2 individuals were grown in a greenhouse in Madison, Wisconsin, and then self-pollinated manually to produce F_3 families.

Experimental design

The 116 F_3 families, parental lines, F_1, and one Group Cantalupensis commercial cultivar ('Sol Dorado'; Syngenta Seeds, Gilroy, CA) were evaluated during the summer for

2 years (2006 and 2007) at the University of Wisconsin Experimental Farm (UWEF), Hancock, WI.

The experimental design employed was a randomized complete block design (RCBD), consisting of three blocks with ten plants per plot. Initially, seeds were sown (Growing Mix No. 2; Conrad Fafard, Inc., Agawam, MA) in a greenhouse (Madison, WI), and then seedlings were transplanted to the field at the three-leaf stage every 0.35 m within rows on 2 m centers (72,600 plants ha^{-1}) into plane field loamy sand (Typic Udipasamment) soil at the UWEF.

Data collection

β-Carotene

Beta-carotene analysis was performed by using the fruit flesh color of each individual plant and the *β*-carotene value of each color grouping (Table 1). First, one mature fruit (i.e., at full slip) was harvested from each plant and cut in transverse section to determine mesocarp (flesh) and endocarp (seed cavity) color of individual plants by visual inspection. Mesocarp and endocarp color was categorized into five and three color groupings, respectively, using the Royal Horticultural Society (2005) mini-color chart. Mesocarp color groupings were orange (ORG; RHS-23C), orange-green (ORGGR; RHS-19B/RHS-149D), light orange (LORG; RHS-18C), green (GR; RHS-145B) and white (WH; RHS-155B or 157B). Likewise, ORG, GR and WH color ratings were utilized for endocarp evaluation. Second, 8–20 fruits from each mesocarp color category provided samples (~5.0 g fresh weight), which were used to determine the Q*β*C in each group by reverse phase high-performance liquid chromatography (HPLC) using a standardized, synthetic *β*-carotene curve (Simon and Wolff 1987). After Q*β*C in each category was determined, mean and standard errors (SE) of each mesocarp color category were calculated, and least significance differences (LSD) among means were determined using SAS (SAS Institute 1999). Subsequently, fruit flesh color data of each individual were used along with their respective color group value (e.g., ORG = 6.62 μg g^{-1} *β*-carotene; Table 1) to determine the *β*-carotene value of each F_3 family, which in fact represents the flesh color segregation within each family.

Fruit maturity

Fruit maturity of an entry was defined as the days from transplanting to the development of the first mature fruit. Plants were evaluated weekly in each of 2 years for maturity during the 6-week harvesting period that began when the first mature fruit was observed in an experiment. Hence, plants were grouped into six maturity categories (weeks 1–6 in the harvesting period; Table 2) for the statistical analysis.

Table 1 Best linear unbiased estimations (BLUE's), number of sample (*n*), and high and low bounds of quantity of *β*-carotene (μg g^{-1}) of commercial melon (*Cucumis melo* L.) hybrids 'Sol Dorado', Chinese line 'Q 3-2-2' (P_1), 'Top Mark' (P_2) and their F_1 progeny ($P_1 \times P_2$)

Cultigen[a]	*n*	BLUE ± SE[b]	High	Low
'Sol Dorado'	10	16.24 ± 0.9	20.09	08.54
'Q 3-2-2' (P_1)	5	00.52 ± 1.1b	00.18	00.08
'Top Mark' (P_2)	10	10.93 ± 1.4a	14.05	07.72
F_1	5	00.77 ± 1.1b	00.71	00.17
Fruit mesocarp color[c]	***n***	**Mean ± SE**	**High**	**Low**
Orange (OR)	16	06.62 ± 0.38a	12.85	02.88
Orange-green (ORGR)	16	04.20 ± 0.60b	06.27	02.22
Light orange (LOR)	8	02.36 ± 0.46c	03.61	01.99
Green (GR)	20	00.25 ± 0.36d	00.94	00.09
White (WH)	20	00.20 ± 0.36d	00.38	00.06

Means, number of sample (*n*), and high and low bounds of quantity of *β*-carotene of fruit mesocarp classifications used to characterize segregating F_3 families evaluated at Hancock, Wisconsin in 2006 and 2007

[a] Refers to line, cultivar, or hybrid

[b] SE = Standard error and different letter indicates values are significantly different at $P \leq 0.05$

[c] Color grouping according to the English Royal Horticultural Society (RHS) mini-color chart (2005), where *WH* white flesh color (RHS-155B), *LORG* light orange flesh color (RHS-18C), *ORG* orange flesh color (RHS-23C), *ORGGR* orange-green flesh color (RHS-19B/RHS-149D), *GR* green flesh color (RHS-145B)

Analysis of variance

Analyses of variance (ANOVA) were performed using the Proc mixed covtest method Type 3 procedure of SAS (SAS Institute 1999). The linear effects model for the analysis was the following: $Y_1 = \mu + Y_2 + B(Y_2) + F + Y_2 \times F + e$, where Y_1 is the trait (i.e., FM or Q*β*C), μ is the common effect, Y_2 is the year effect, $B(Y_2)$ is the block within year effect, F is the effect of F_3 families, $Y_2 \times F$ is the year × F_3 family interaction effect, and e is the plant-to-plant variation within F_3 families.

Best linear unbiased predictors (BLUPs; Bernardo 2002), SE and 95% confidence intervals (CIs) were estimated for each F_3 family using the solution option of the random statement of the proc mixed covtest procedure in SAS (SAS Institute 1999). Best linear unbiased estimators (BLUEs) were also estimated for P_1, P_2, F_1 and 'Sol Dorado' using the solution option of the model statement of the proc mixed covtest procedure (SAS Institute 1999). This procedure estimates fixed effects values from the raw data while making variable value adjustments during such estimations (de Leon et al. 2005).

Table 2 Means and standard error (SE), and number of samples (*n*) for fruit maturity (full slip) in commercial melon (*Cucumis melo* L.) hybrid 'Sol Dorado', Chinese line 'Q 3-2-2' (P_1), 'Top Mark' (P_2) and their F_1 progeny given as a range of numbers of days from transplanting to the development of the first mature fruit, and ranking used to characterize segregating F_3 families evaluated at Hancock, Wisconsin in 2006 and 2007

Cultigen[a]	*n*	2006	2007	Rank[b]	2006	2007
'Sol Dorado'	10	094 ± 2	101 ± 1	1	68–74	73–79
'Q 3-2-2' (P_1)	5	072 ± 2	078 ± 1	2	75–81	80–86
'Top Mark' (P_2)	10	100 ± 1	106 ± 1	3	82–88	87–93
F_1	5	082 ± 2	082 ± 1	4	89–95	94–100
				5	96–102	101–07
				6	103–109	108–115

[a] Line, cultivar or hybrid

[b] Ranking refers to days from transplanting to the development of the first mature fruit (full slip)

To assess whether significant genotype × environment (G × E) interactions were due to trait magnitude changes between years or changes in the direction of the response (i.e., F_3 family rank changes), Spearman (rank) correlation coefficients (r_s) were calculated using F_3 family data for FM across years according to Yan and Rajcan (2003). When the correlation coefficient (r_s) between data across year was $r_s \leq 0.5$, G × E interactions were considered more likely to be due to F_3 family rank changes, and when $r_s \geq 0.5$, such interactions were considered more likely to be due to trait magnitude changes between years.

Phenotypic and genetic correlation

Phenotypic correlations between FM and QβC in F_3 families were calculated as Spearman coefficients (SAS Institute 1999). The genetic correlations and their attending standard errors were calculated according to Falconer and Mackay (1996).

PCR amplification of putative carotenoid structural and related genes

Previously published degenerative primers (Cuevas et al. 2008) for nine carotenoid structural genes [Phytoene synthase (PS), beta-carotene hydroxylase-1 (BOH-1), beta-carotene hydroxylase-2 (BOH-2), lycopene β-cyclase (LycB), carotenoid isomerase (CRTISO), zeaxanthin epoxidase (ZEP), zeta carotene desaturase (ZCDS), phytoene desaturase (PDS) and violaxanthin de-epoxidase (VDE)] and the orange (Or) related gene were utilized to amplify parental DNA (Chinese line 'Q 3-2-2' and 'Top Mark'). Polymerase chain reactions (PCR) and cycling conditions were performed according to Zalapa et al. (2007), and annealing temperatures were according to Cuevas et al. (2008). After gel electrophoresis, unique amplicons were physically isolated, purified using the Wizard SV Gel clean-up system (Promega Corp., Madison, WI) and subsequently sequenced via BigDye terminator chemistry. Parental line sequence polymorphisms were examined using the Staden Package software, and then more specific primer sets flanking each polymorphism were designed (Table 3).

Melon SSR and cucumber EST evaluation

A total of 492 cucurbit [squash (*Cucurbita* ssp.), melon and cucumber (*C. sativus* L.)] SSR (31, Chiba et al. 2003; 144, Ritchel et al. 2004; 183, Fukino et al. 2007; 57, Gonzalo et al. 2005; 34, Danin-Poleg et al. 2000; 15, Fazio et al. 2002; and 28, Fernandez-Silva et al. 2008), 20 EST-SSR markers of cucumber (Kong et al. 2006) and 22 EST-SSR markers of melon (Kong et al. 2007) were used to amplify parental DNA for polymorphism detection according to Zalapa et al. (2007). A parental screen was performed using capillary electrophoresis employing fluorescent dUTP (ChromaTide Alexa Fluor 546-14-dUTP, Invitrogen, Carlsbad, CA) in the PCR reaction, and GeneMarker V1.5 software was used to determine alleles' sizes (Soft Genetics LLC-2005, State College, PA). The genotyping of F_2 individuals (used to develop F_3 families) was performed using 4% (w/v) Metaphor agarose (Lonza, NJ) or capillary electrophoresis, depending on allelic size differences.

A total of 53 unpublished cucumber EST primers (Johnson, personal communication Madison, WI, 2008), which produced unique amplicons in melon (Cuevas et al. 2008) were also used to amplify parental DNA for polymorphism detection. Amplicons were purified using the Wizard SV Gel clean-up system (Promega Corp., Madison, WI), and sequenced via BigDye terminator chemistry. Single nucleotide polymorphisms (SNP) between parental lines were identified and then utilized to develop cleaved amplified polymorphic sequences (CAPS) markers using appropriate

Table 3 Carotenoid structural and related genes mapped in melon (*Cucumis melo* L.) using 116 F_2 progeny derived from a cross between Chinese line 'Q 3-2-2' (P_1) and 'Top Mark' (P_2)

Enzyme name	Gene symbol	Contig ID of Melon EST database	Primer sequences used for genotyping and sequencing	Annealing temperature (°C)	Fragment size (bp)	Polymorphism
β-Carotene hydroxylase[a]	BOH-1	MU9511	F-TGGGCVIGRTGGGCBCA	60	523	SNP[b]
			R-TCCCCAGTGATTAAACAAACA			
Lycopene β-cyclase	LycB	MU9517	F-TCAAGCTGCCATTGTTCTTG	60	544	SNP[b]
			R-AACGTCCATCTGAACCAAGG			
Zeaxanthin epoxidase[a]	ZEP	MU7815	F-TCACATTGTCACACCAACCA	60	430	SNP[b]
			R-AAACACCCCAATCAACCAGA			
Phytoene desaturase	PDS	MU9070	F-TCCTGCACCCATAAATGGTA	60	699	SNP[b]
			R-TCCAGTTTATGTTACCCGCATA			
Violaxanthin de-epoxidase	VDE	–	F-TCCAAAATGTTCGTTTTGACC	60	700	SNP[b]
			R-AAGGAGGCCAACTTGAAATG			
Orange gene	Or	MU4014	F-AAGCTACGGTCCCATTTGGT	60	325	INDEL (17 bp)[c]
			R-TGGCAATTGAAATTTTGAAAGA			

[a] Primers from Cuevas et al. (2008)

[b] SNPs genotyped with direct sequencing using specific primers

[c] Insertion/deletion (INDEL) used to construct a co-dominant marker visualized by agarose gel electrophoresis

restriction enzymes (RE) (Parsons and Heflich 1997). After performing specific primer-based PCR, 5 µl of the recovered amplicons was mixed with 15 µl containing one unit of RE and buffer supplied by the manufacturer, and this solution was then incubated for 2 h at temperatures that optimized RE. The digestion products were then evaluated by electrophoresis using 1% (w/v) agarose, stained with ethidium bromide and photographed using a Dark Reader™ transilluminator (Clare Chemical Inc., Denver, CO.).

Linkage map construction

The degree of disturbed segregation (significance declared at $P < 0.01$) associated with SSR, CAPS and SNP markers were determined by marker data comparison against the expected 1:2:1 ratio for an F_2 population using Chi-square analysis (Vuylsteke et al. 1999). Genotypic data produced a matrix of 169 co-dominant markers (154 SSR, 8 CAPS, and 7 SNP) for analysis.

A linkage map was constructed using MapMaker/EXP 3.0 (Lander et al. 1987), where markers were associated with the group command at LOD = 5.0 and a recombination frequency value of 0.30. Markers within a group were ordered using the Order command at LOD of 3.0. The remaining markers were then located with the Try command, and the map order was re-tested using the Ripple command. Map distances were calculated using the Kosambi function.

QTL mapping

Composite interval mapping (Zeng 1993, 1994) was performed using Windows QTL Cartographer 2.5 (Wang et al. 2001–2004) with a walking speed of 1 cM and a window size from 0.5 to 5.0 cM, where up to 12 maximum background marker loci were selected by stepwise forward regression to reduce background effects. A QTL was declared significant when the LOD score was higher than the LOD threshold calculated using 1,000 permutations with an experimental-wise (type I) error rate of $P = 0.05$ for both traits. The QTL nomenclature for QβC was designated as β-carM or β-carE, where M and E refer to QβC in mesocarp and endocarp (seed cavity), respectively, and FM for fruit maturity, preceding their linkage group and locus designation (e.g., β-carM.9.1).

Two-dimensional genome scans for the detection of epistatic interactions were performed by employing the Haley–Knott protocol (HK; Haley and Knott 1992) in R/qtl (Broman et al. 2003), which identifies putative epistatic interactions by pair-wise comparison. The multi-point genotype probabilities for the HK analysis were calculated using the *Calc.genoprob* command with a step interval of 2 cM and error probability of 0.01. Two-dimensional genome scans calculated LOD scores for the full (LOD_{FULL}; two QTL plus interaction) and additive (LOD_{ADD}; two QTL but not interaction) model, which were then used to calculate interaction LOD scores (LOD_{INT}) by subtracting LOD_{FULL} value from LOD_{ADD} value (i.e.,

$LOD_{INT} = LOD_{FULL} - LOD_{ADD}$). The LOD threshold for the interaction was determined by 1,000 permutations and an experimental-wise error rate (type I) greater than $P = 0.05$ for both traits. In addition, independent QTL identified by composite interval mapping were evaluated for interaction with linear regression analysis using the *fix.qtl* command, where the linear model was $Y = QTL + QTL_{INTERACTION}$, and Y refers to QβC or FM.

Map merging

A previously published linkage map, constructed using RIL ($n = 81$), derived from a cross between 'USDA 846-1' × 'Top Mark' [Cuevas et al. 2008; composed of 104 SSR, 7 CAPS, 4 SNP, and 140 dominant markers (random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP)] was visually compared to the F_2 linkage map ('Q 3-2-2' × 'Top Mark') created using common SSR and SNP markers. Common markers were required to have the same recombination fraction (rf) among populations for use as anchor makers in map merging (Liu 1998). Therefore, the utility of 59 common markers (distributed across 12 linkage groups) as potential anchor markers was evaluated using the heterogeneity test available in Joint Map software (Stam 1993) to determine if they possessed the same recombination fraction in both populations (RIL and F_2). Common markers with differing recombination fraction (significance declared at $P < 0.05$) were excluded for use as anchor markers. Subsequently, the two maps were merged using Joint Map software (LOD = 2.0; rf = 0.35–0.50) by employing the "*fixed order*" option according to Qi et al. (1996). This option allows for definition of fixed order marker subsets based on marker order in individual linkage groups, which consequently results in optimal marker order (i.e., lower likelihood value).

Results

β-Carotene analysis and fruit maturity

Differences in orange fruit colors of the mesocarp were associated with differences in QβC (Table 1). Regardless of the growing environment (i.e., year), there were significant differences ($P < 0.05$) detected among the three orange color categories (ORG, ORGGR, and LORG) and associated QβC (6.62, 4.20, and 2.36 μg g^{-1}, respectively). This was not the case between green and white mesocarp phenotypes (0.25 and 0.20 μg g^{-1}, respectively). Growing environment affected maturity timing in the beginning of the harvest period, where the time to first harvest in 2007 was 6 days later than in 2006 (Table 2). Nevertheless, the total harvest period length was 6 weeks in both years.

Analysis of variance

The F_3 family-based ANOVA indicated significant main effects for families ($P \leq 0.001$) and growing environment (years; $P \leq 0.05$) for QβC and FM, while year × family interactions were significant ($P \leq 0.001$) only for FM. Spearman correlations (r_s) for FM indicated that the interaction between year and F_3 families was due to changes in trait magnitude (i.e., not to F_3 family rank changes) in the 2 years examined ($r_s = 0.82$).

Parental trait distributions represented the extreme values for the two traits examined (Table 1, 2). Although the parents differed ($p \leq 0.001$) in FM and QβC in both years, year × genotype interactions between parents for these traits were not significant. Moreover, F_3 family values did not approach the upper or lower parental extremes for FM and QβC in each year, and phenotypic and genetic correlations between FM and QβC were not significant.

Carotenoid-related genes

Genomic sequences from nine structural carotenoid genes and one carotenoid-related gene were analyzed using previously published degenerative primers (Cuevas et al. 2008). Polymorphisms were identified in five structural carotenoid genes (BOH-1, LycB, ZEP, PDS, and VDE; Table 3) and the carotenoid-related gene (Or), and were used to construct the F_2-based map presented. Three structural carotenoid genes (BOH-1, ZEP, and VDE) were previously mapped using an RIL population (Cuevas et al. 2008), and thus were employed for synteny analysis with the F_2 map constructed (Fig. 1).

SSR evaluation and cucumber EST

Parental screening identified 155 (~32%) polymorphic markers in previously reported SSR arrays [Chiba et al. (2003; 7), Ritchel et al. (2004; 46), Fukino et al. (2007; 44), Kong et al. (2006), 2007; 11), Fazio et al. (2002; 3), Gonzalo et al. (2005; 33) and Fernandez-Silva et al. (2008; 11). While 81 (52%) of these SSR markers have been previously mapped (Perin et al. 2002; Silberstein et al. 2003; Gonzalo et al. 2005; Fukino et al. 2008, Cuevas et al. 2008 and Fernandez-Silva et al. 2008), 75 markers were unique to the map construction. Although two markers [GCM548 (LG II) and GCM181 (LG VII)] showed distorted segregation ($X^2 = 9.4$, $P < 0.009$; and $X^2 = 10.1$, $P < 0.006$, respectively), GCM548 was included, since its map position was comparable to another melon map (Fernandez-Silva et al. 2008). Single nucleotide polymorphisms were identified in nine (CU2578, CU2527, CU6, CU160, CU491, CU2186, CU2484, CU2557, and CU2544) cucumber EST primers (~17%). While eight markers did not exhibit segregation

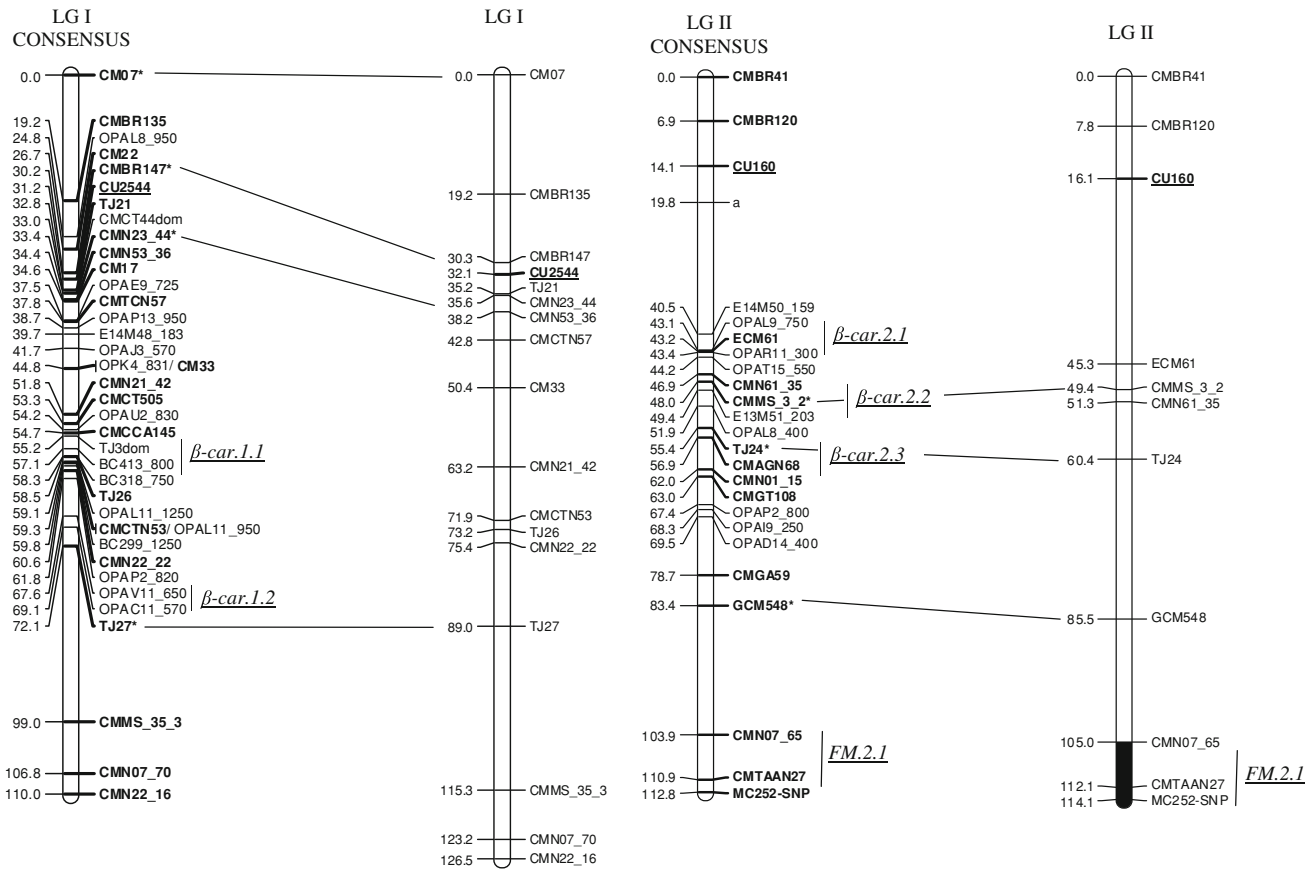


Fig. 1 Consensus (*left*) and component (used to derive consensus map; *right*) maps depicting locations of quantitative trait loci (QTL) associated with quantity of β-carotene (β-car.) and fruit maturity (FM.) in melon (*Cucumis melo* L.). Component map was constructed using an F_2 (116) population derived from a cross of Chinese line 'Q 3-2-2' × 'Top Mark'. Consensus map was constructed from the F_2 map and a recombinant inbred line (81 lines) map derived from a cross of 'USDA 846-1' x 'Top Mark' (Cuevas et al. 2008), where linkage groups are designated according to Perin et al. (2002). Co-dominant markers are in *bold* in consensus map. Interspecies markers from cucumber (*Cucumis sativus* L.) are *underlined* and in *bold*. Carotenoid and related genes are *italicized* and in *bold* in consensus and component map. *Asterisks* and *lines* refers to anchor markers used during map merging of the two component maps

distortion, analysis of CU2186 (LG IV) revealed distorted segregation ($X^2 = 17.1$, $P < 0.001$), and was thus not included in map construction. While five cucumber EST have been previously mapped (Cuevas et al. 2008), four of these markers were unique to the map construction. Comparative analysis of these nine polymorphic cucumber EST sequences revealed that CU2578, CU6, CU160, CU491, CU2186, CU2484, CU2557 and CU2544 were homologous to melon EST sequences, MU16153, MU10410, MU3556, MU15482, MU9093, MU15536, MU4718 and MU4748, respectively.

Linkage map construction

The 169 co-dominant markers (154 SSR, 8 CAPS, and 7 SNP) employed for linkage analysis resulted in the construction of a map consisting of 13 linkage groups (LG). One of these LG consisted of three markers (CU6, CMN_B10 and CMBR154; spanning 3.2 cM) and was identified as a part of LG IV (Cuevas et al. 2008). Thus, the map presented consists of 12 LG spanning 1,095 cM, with a mean marker interval of 6.5 cM where the largest distance between any two markers was 31.9 cM (CM5 and CM26 locus; LG VII, Fig. 1).

Map integration

The RIL-based map of Cuevas et al. (2008) and the F_2 map constructed were used in map merging experiments. Two linkage groups [LG III (CU2578) and LG V (VDE)] possessed only a single common marker between the maps, and thus optimum alignment and subsequent integration could not be achieved. Nevertheless, 50 common anchor markers distributed across 10 linkage groups (Fig. 1) were employed for map merging.

The consensus map that was constructed consisted of 299 points [298 molecular markers and a morphological marker (a)] spanning 10 LG covering 942 cM (Fig. 1). Marker type integral to the map varied to include 185

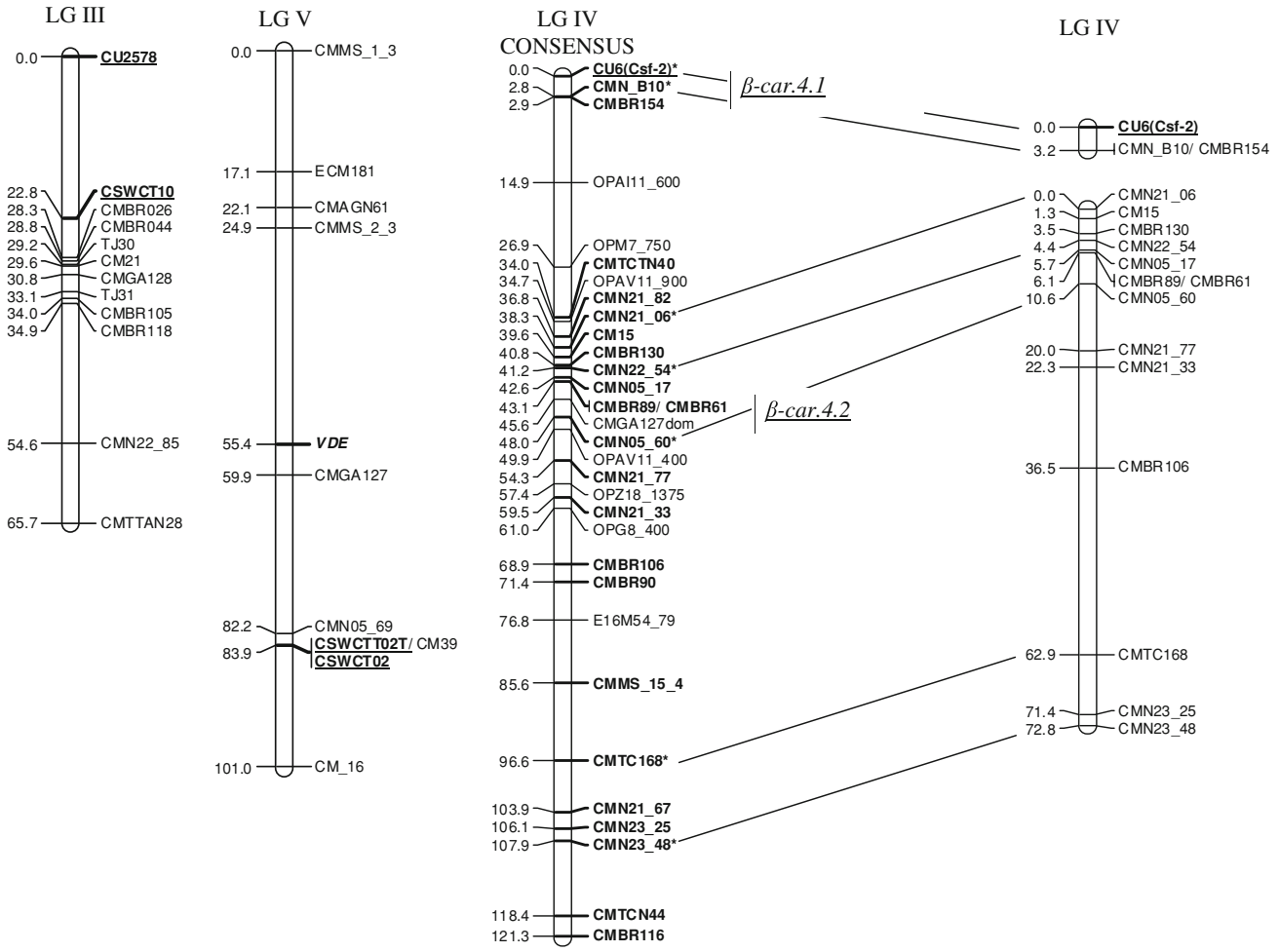


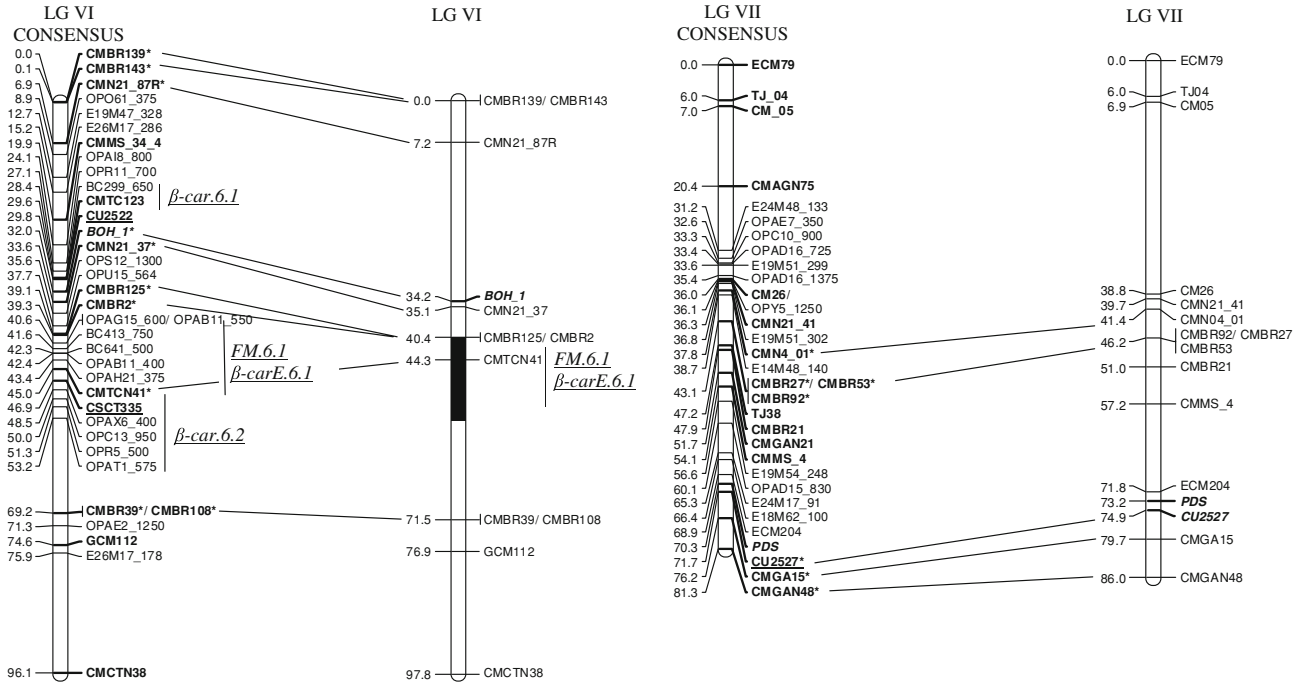
Fig. 1 continued

(62%) co-dominant [158 SSR-Melon, 9 EST-SSR-Melon, 5 carotenoid genes and 13 interspecies markers from cucumber (*Cucumis sativus* L.; 4 SSR-cucumber, 8 EST-cucumber and 1 EST-SSR-cucumber] and 113 (38%) dominant (88 RAPD and 25 AFLP) loci. The mean marker interval in the merged map was 3.6 cM, and the largest distance between any two markers was 26.9 cM (TJ27 and CMMS35_3 locus; LG I). Although the creation of a consensus map increased map saturation (27%), eight relatively large genomic gaps (>15 cM) could not be saturated in the consensus map in LG I, LG II, LG VI and LG XI. Moreover, even though marker order among the three maps (consensus, F_2 and RIL) was reliable, some slight differences were observed between the consensus and complements maps (F_2 and RIL). However, such inconsistencies were observed in closely linked markers (<10 cM).

QTL analysis

Fruit mesocarp color was classified as ORG, ORGGR, LORG, GR and WH, and endocarp (seed cavity) color was classified as ORG, GR and WH in segregating F_3-cross-progeny families. Thus, QTL analyses were performed separately for mesocarp and endocarp tissue. The mean of QβC ($\mu g\ g^{-1}$) was consistently associated with color scores [i.e., ORG (6.62), ORGGR (4.20), LORG (2.36), GR (0.25), and WH (0.20); Table 1] and, therefore, QTL identified were related to variation in flesh color.

Composite interval mapping detected two QTL for QβC in mesocarp located in two LG [LG VIII (β-carM.8.1), and LG IX (β-carM.9.1)] (Table 4; Fig. 1). Likewise, two QTL were detected for QβC in endocarp located in two LG [LG VI (β-carE.6.1) and LG IX (β-carE.9.1)], where the QTL on LG IX (β-carE.9.1) resided at the same position as a QTL (β-carM.9.1) for mesocarp tissue (i.e., 3 QβC-associated QTL). All QβC-associated QTL detected were defined well above the mapping threshold ($P \leq 0.001$; LOD > 5.7), and the proportion of the phenotypic variance explained by a single QTL (R^2) ranged from 4.0% (β-carE.6.1) to 50.0% (β-carE.9.1). The QTL β-carM.9.1/β-carE.9.1 explained a significant portion of the phenotypic variance ($R^2 > 40\%$) and thus must be considered as major QTL. All the additive effects for QβC



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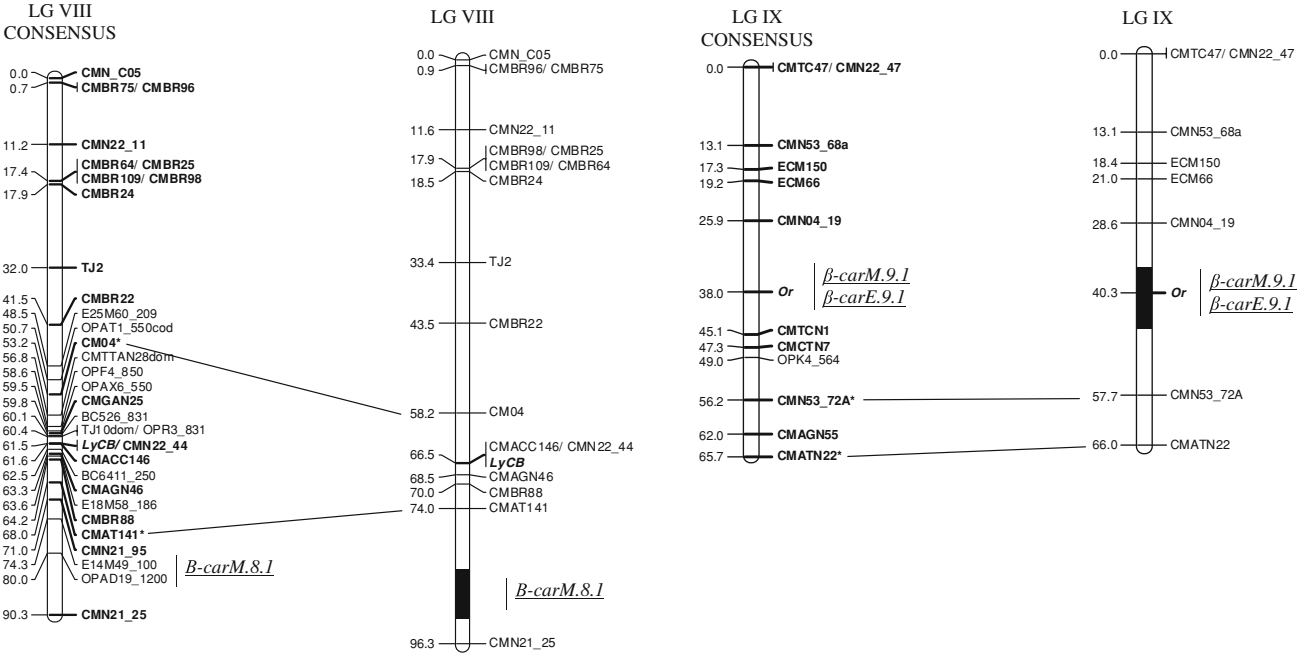

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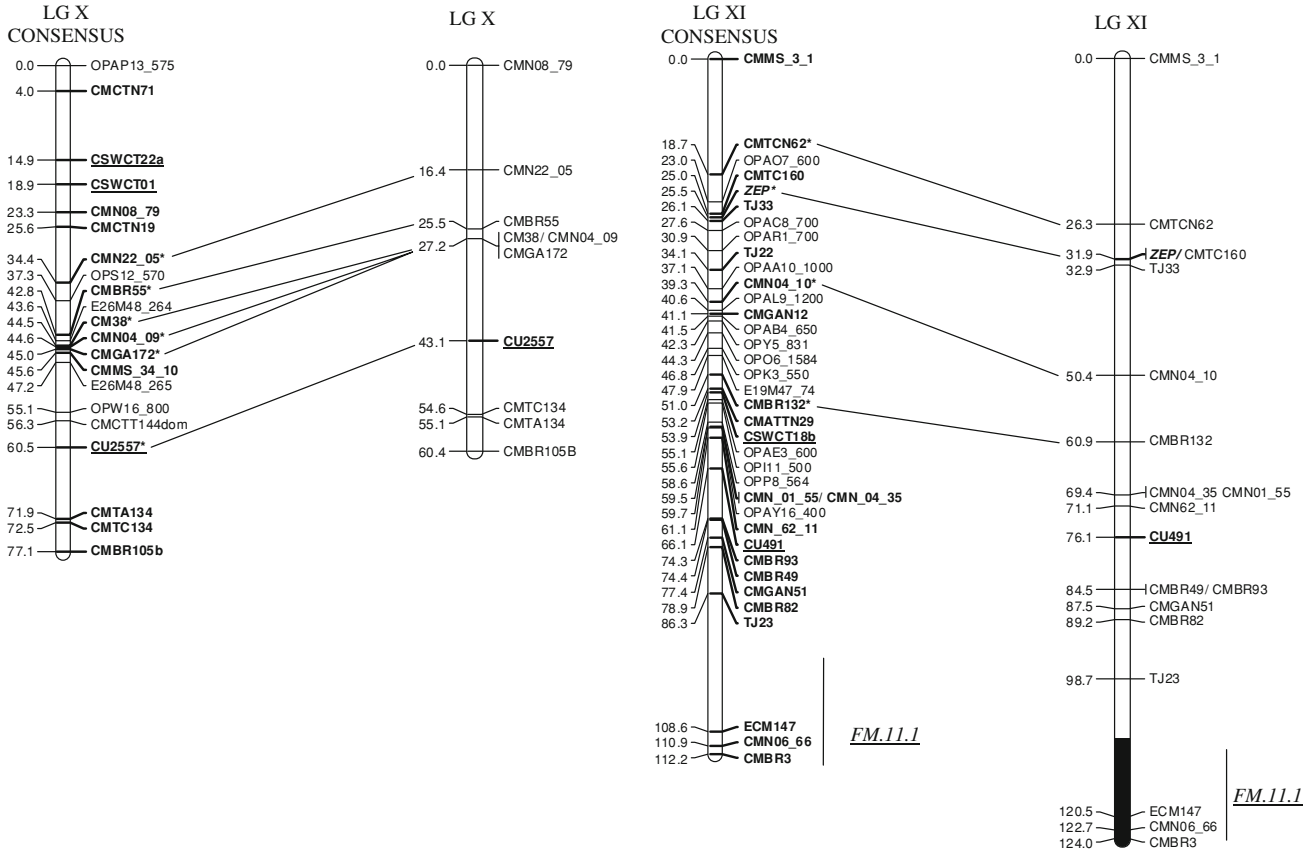
were contributed by 'Top Mark' alleles. However, the contribution of dominance effects varied depending on specific QβC-QTL. For instance, dominance effects of some QTL (e.g., β-carM.8.1, β-carM.9.1 and β-carE.6.1) were contributed by alleles resident in line 'Q 3-2-2', while others (e.g., β-carE.9.1) were specific to alleles in 'Top Mark'.

Three QTL for FM (FM.2.16, FM.6.1 and FM.11.1) were detected ($P \leq 0.05$; LOD > 4.11) in three LG (LG II, LG VI and LG XI) (Table 4; Fig. 1). The QTL FM.6.1 in LG VI (LOD = 14.3; $R^2 = 35\%$) and FM.11.1 in LG XI (LOD = 8.6; $R^2 = 20\%$) must be considered as major QTL, since they explained a significant proportion of the phenotypic variance. All additive genetic effects for maturity of fruit were contributed by 'Top Mark' alleles, and all dominance effects were contributed by line 'Q 3-2-2' alleles.



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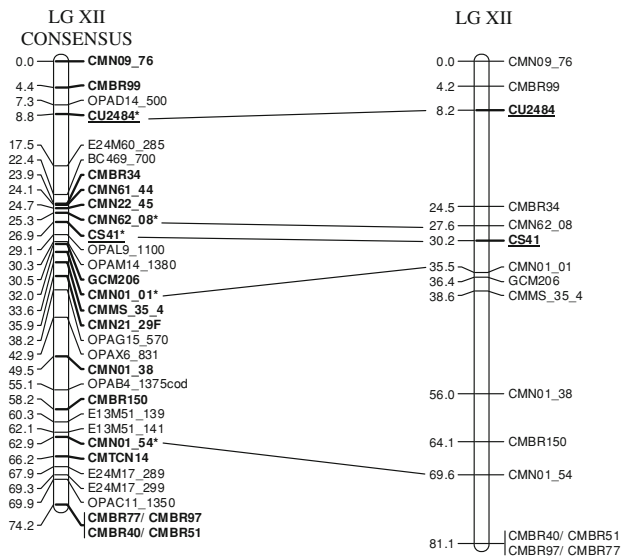

Fig. 1 continued

Two-dimensional epistasis genome scans

No epistatic interactions were detected for either QβC or FM in the 96 pair-wise genome comparisons investigated (data not presented). However, significant putative epistatic interactions were identified between the independent QTL identified with composite interval mapping analyses (Table 5). While interactions were detected between fruit mesocarp QTL β-carM.9.1 and β-carM.8.1 ($p < 0.01$), endocarp QTL β-carE.9.1 was found to interact with β-carE.6.1 ($p < 0.05$). These interactions explain 4.4 and 0.7% of the phenotypic variance of QβC present in fruit mesocarp and endocarp, respectively. Moreover, linear regression analysis employing all main and interaction QTL effects for QβC was significant ($P \leq 0.05$), explaining 77.7 and 94.7% of the phenotypic variation observed in the mesocarp and endocarp tissue, respectively. In contrast, putative epistatic interactions between QTL associated with the maturity of fruit, as detectable by composite interval mapping analyses, were not identified in this analysis.

Discussion

Concentrations of QβC can range from 9 to 18 $\mu g\ g^{-1}$ in fresh mesocarp fruit tissue of orange melon commercial varieties (Navazio 1994). Although QTL associated with QβC (intensity of orange color) have been recently identified

Table 4 Quantitative trait loci (QTL), linkage group position, associated logarithm of odd (LOD), percentage of explained phenotypic variation (R^2), additive and dominance effect for quantity of β-carotene (QβC; flesh color) and fruit maturity in melon (*Cucumis melo* L.) using 116 F_3 families from a cross of Chinese line 'Q 3-2-2' × 'Top Mark' evaluated in Hancock, Wisconsin in 2006 and 2007

Trait	QTL[a]	Linkage group	Position (cM)	Nearest marker locus[b]	LOD	R^2 (%)	Additive effect[c]	Dominance effect[c]
QβC mesocarp	β-carM.8.1	VIII	87.00	CMN21_25	7.81	11.0	0.51	−0.39
	β-carM.9.1	IX	37.60	Or	23.40	40.0	1.37	−0.67
QβC endocarp	β-carE.6.1	VI	40.11	CMTCN41	7.27	4.0	0.59	−0.07
	β-carE.9.1	IX	41.30	Or	49.70	50.0	2.59	0.70
Fruit maturity[d]	FM.2.1	II	100.50	CMN07_65	4.46	8.0	0.23	−0.03
	FM.6.1	VI	50.30	CMTCN41	14.28	35.0	0.53	−0.13
	FM.11.1	XI	112.70	TJ147	8.55	20.0	0.47	−0.27

[a] QTL designated by abbreviated trait name, linkage group name and QTL number

[b] Nearest marker to peak of the detected QTL

[c] Additive and dominance effect as obtained from a composite interval mapping (CIM) model resident in QTL cartographer (Wang et al. 2004) as represented by the effect associated with 'Top Mark'

[d] Maturity is defined as the days from transplanting to the development of the first mature fruit

Table 5 Regression analysis of quantitative trait loci (QTL) associated with the quantity of β-carotene (QβC; flesh color), as defined by segregation in 116 F_3 families derived from a cross between Chinese line 'Q 3-2-2' (P_1) and 'Top Mark' (P_2) grown at Hancock, Wisconsin in 2006 and 2007

QTL[a]	df[b]	SS[c]	R^2 (%)[d]	p-Value (X^2)[e]	p-Value (F)[e]
Mesocarp tissue					
β-carM.8.1	6	23.03	12.57	***	***
β-carM.9.1	6	110.85	60.54	***	***
β-carM.8.1 × β-carM.9.1	4	7.98	4.36	**	**
Endocarp tissue					
β-carE.6.1	6	16.10	3.31	***	***
β-carE.9.1	6	440.54	90.70	***	***
β-carE.6.1 × β-carE.9.1	4	3.17	0.70	*	*

Regression analysis was performed using R/qtl (Broman et al. 2003)

[a] QTL identified using composite interval mapping (CIM) model in QTL cartographer (Wang et al. 2004), and designated by abbreviated trait name, linkage group name and QTL number

[b] *df* Degrees of freedom

[c] Sum of squares

[d] Percentage of explained phenotypic variation

[e] *, **, *** Effect is significant at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$, respectively

(Cuevas et al. 2008) in the USWS melon type, the genetics of FM has not been studied. In fact, early maturating germplasm with enhanced QβC (>25 μg g^{-1}) is not publicly available. In this regard, the exotic Chinese germplasm 'Q 3-2-2' is an early maturing cultivar whose genes could be readily introgressed into USWS market types. However, this process could be affected or limited by the presence of white flesh genes in Chinese germplasm. The characterization of QTL associated with flesh color and FM in a cross between Chinese germplasm and US Western Shipping market class, and increased map saturation by map merging [with the Cuevas et al. (2008) map] described herein will allow for the development of marker-assisted selection (MAS) breeding strategies to increase gain from selection (ΔG) for QβC, FM and other traits in different melon genetic backgrounds.

Linkage map and map integration

A saturated map of melon has been estimated to have a total length of 1,500–2,000 cM across 12 LG (Baudracco-Arnas and Pitrat 1996; Perin et al. 2002). However, recently published maps having 12 LG ranged from 900 to 1,200 cM in length (Gonzalo et al. 2005, Fukino et al. 2008; Cuevas et al. 2008). The RIL-based ('USDA 846-1' × 'Top Mark'; Cuevas et al. 2008) map utilized for map merging (designated as a component map) consisted of 12 LG, with a mean marker interval of 4.6 cM and a total length of 1,180 cM. Correspondingly, the F_2-based ('Q 3-2-2' × 'Top Mark') component map possesses 12 LG and spans 1,095 cM, with a mean marker interval of 6.5 cM. The similarity in length of these two maps and other melon published maps (Gonzalo et al. 2005, 1,021 cM; Fukino et al. 2008, 900 cM) provides confidence that the total length of melon map is 1,000–1,500 cM spanning 12 LG. Although two linkage groups (LG III and LG V) could not be integrated since they possessed only a single common marker, the colinear marker order among the RIL, F_2 and consensus maps documented provides confidence in marker order reliability given the anchor markers used.

The total length of the consensus map (942 cM; 10 LG) was slightly smaller than the RIL-based map (997 cM; 10 LG), and larger than the F_2-based map (928 cM; 10 LG).

Some marker order differences have been observed during map merging experiments, especially where markers are tightly linked (*Arabidopsis*; Hauge et al. 1993; Somers et al. 2004). Moreover, varying recombination rates between two mapping population [e.g., RIL (S_{7-8}) vs. F_2 used] can lead to marker order differences during map merging (Malippeard et al. 1998). In this study, marker order and position among the RIL and F_2 maps were comparable, except for markers CM04 in LG VIII and CMTCN41 in LG VI. Since locus order was determined using data collected from relatively small populations (i.e., 81 RIL and 116 F_2 individuals), position differences observed in these two markers could be attributable to sampling error (Liu 1998).

Moderately saturated genetic maps exist for melon (Baudracco-Arnas and Pitrat 1996; Perin et al. 2002; Gonzalo et al. 2005; Cuevas et al. 2008; Fukino et al. 2008) and those incorporating SSR markers have been proposed for map merging experiments (Gonzalo et al. 2005). Where common QTL for horticultural traits have been defined, comparative mapping can be performed in diverse cucurbit mapping populations (Staub et al. 2007). However, such intra-species map comparisons can be compromised by inadequate numbers of syntenic markers (Moore et al. 1993). Although the RIL and F_2-based maps that were used incorporated a significant number of SSR markers (65 and 55, respectively) common to previously published maps (Gonzalo et al. 2005; Fukino et al. 2008), some LG regions lacked adequate SSR numbers for QTL genome comparisons. Nevertheless, the two maps merged in the present study ('Top Mark' as a common parent) spanned several complementary unsaturated genomic regions resulting in a consensus map (996.8 cM; 10 LG), which was potentially valuable for comparative genomic analyses.

The consensus map described affords a 30% increase (i.e., 67 additional co-dominant markers) in the number of markers over a previously published map on USWS melon (Cuevas et al. 2008). This level of increased saturation allows for comparative genome location analyses of QTL associated with yield (Zalapa et al. 2007), fruit quality components (Paris et al. 2008) and nutritional value (Cuevas et al. 2008) to further define QTL interactions and provide for more complete trait dissection.

Carotenoid genes and β-carotene accumulation

The association between carotenoid biosynthesis genes and QTL has been documented in maize (*Zea* spp.; Wong et al. 2004), wheat (*Triticum* spp.; Pozniak et al. 2007) and pepper (*Capsicum* spp.; Throup et al. 2000; Huh et al. 2001). In contrast, QTL studies on tomatoes (*Solanum* spp.; Fulton et al. 2000), carrots (*Daucus* spp.; Santos and Simon 2002; Just et al. 2007) and melon (Cuevas et al. 2008) have not identified associations with biosynthesis carotenoid genes. Moreover, although the action of several genes [e.g., Or (cauliflower; *Brassica oleracea*) and DEETIOLATED1 (tomato; *Solanum lycopersicum*] are associated with carotenoid accumulation (Lu et al. 2006; Davuluri et al. 2005, respectively), the biochemical activity of these genes is not directly related to the carotenoid biosynthesis pathway.

The map positions of the Qβ C-associated QTL identified were not closely aligned with the position of six mapped biosynthesis carotenoid genes. However, the QTL interval of β-carE.6.1 is located 10 cM from the β-carotene hydroxylase-1 gene (BOH-1; Fig. 1), and is syntenic with QTL identified in RIL (Cuevas et al. 2008). Moreover, a major QTL identified, β-carM/E.9.1 (βcarM.9.1, LOD = 23.4, $R^2 = 40.0\%$ and β-carE.9.1, LOD = 49.7, $R^2 = 50.0\%$), showed alignment with Or, an unrelated carotenoid biosynthesis pathway gene associated with Qβ C accumulation in cauliflower (Lu et al. 2006). The Or gene regulates the synthesis of a DnaJ cysteine-rich-domain protein (e.g., chaperon protein) that in turn triggers proplastid and/or other non-colored plastid differentiation into chromoplast plastids, which then provides for a "metabolic sink" for carotenoid accumulation (Lu et al. 2006). Syntenic relationships among the present consensus map and other melon maps (Perin et al. 2002 and Fukino et al. 2008) indicates that βcarM/E.9.1 and the Or gene map to the same genomic region as wf gene (white flesh; Imam et al. 1972, Fig. 2). Although the mapping interval associated with the Or gene is large (30 cM), it is hypothesized that this may be a major gene associated with fruit mesocarp color in melon and perhaps directly related to the previously reported white flesh gene (wf; Imam et al. 1972). This hypothesis could be more rigorously evaluated when marker saturation in this region increases to allow for fine mapping and accompanying sequence analysis.

The green mesocarp color gene, gf (Hughes 1948), was mapped to the distal end of LG I (distal to SSR CMAT141) by Monforte et al. (2004), which is synonymous with LG VIII of Eduardo et al. (2007). However, near isogenic lines developed from the same mapping population located the region containing gf proximal to CMAT141 (Eduardo et al. 2007). Comparative mapping employing SSR markers common to this region of LG VIII indicates that Qβ C-QTL β-carM.8.1 is, in fact, located distally to SSR CMAT141 (Fig. 1), which is in agreement with Monforte et al. (2004).

Genetic control of Qβ C and early fruit maturity

The analysis of mesocarp color segregation in F_2 progeny (parental to the F_3 families used) indicated that flesh color variation is under the control of two major epistatic genes (white:green:orange; 9:3:4; $X^2 = 1.6$, $P = 0.45$). However, segregation within F_3 families could not support this model, suggesting that these two major genes interact with minor

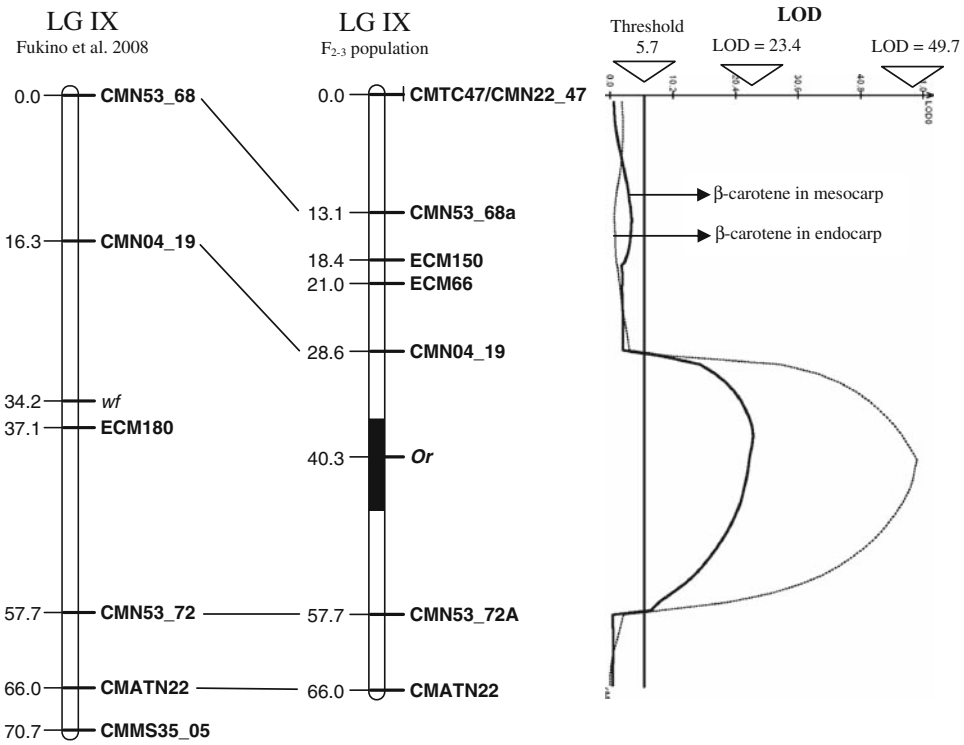

Fig. 2 Colinearity and synteny relationships between Linkage Group IX constructed using recombinant inbred lines derived from a cross between 'AR 5' (orange fleshed) × 'Harukei 3' (green fleshed) [Fukino et al. 2008; mapping of white flesh gene (wf; Imam et al. 1972)], and an F_{2-3} population derived from a cross between Chinese line 'Q 3-2-2' (white fleshed) and 'Top Mark' (orange fleshed) as used to map Or gene (Lu et al. 2006) and QTL associated with β-carotene (i.e., orange flesh) in melon (*Cucumis melo* L.)

genes to produce orange hues within the ascribed color categories (ORG, ORGGR, and LORG). The QTL analyses performed support this hypothesis since one major QTL (β-carM/E.9.1; LOD = 23.4/49.7) defines ~50% of the phenotypic variation associated with fruit mesocarp color variation and interacts with minor QTL (β-carE.6.1 and β-carM.8.1) to provide color development in this population (Tables 4, 5). The phenotypic fruit data collected (five and three color classes in mesocarp and endocarp, respectively) did not allow for fine discrimination of color variation (i.e., hues) suggesting that additional minor QTL for this trait may exist. Hence, this QTL analysis is complementary to that of Cuevas et al. (2008) where five QβC-associated QTL (intensity of orange color) were detected. The colinearity between the two maps used for consensus map construction allowed for fruit color QTL comparisons, which suggested that QTL β-carE.6.1 ('Q 3-2-2' × 'Top Mark') are likely equivalent to β-car.6.2 ('USDA 846-1' × 'Top Mark'; Cuevas et al. 2008). The fact that these QTL are syntenic with a major orange flesh color QTL (ofc12.1) identified by Monforte et al. (2004) supports this contention.

The inheritance of FM in melon is complex and likely involves many genes. Two major QTL (FM.6.1 and FM.11.1; $R^2 = 35$ and 20%, respectively) and one minor QTL were detected (Table 4; Fig. 1). Genetic theory (Lande and Thompson 1990) and computer simulation studies (Beavis 1994) suggest that the precision of QTL detection is a function of mapping population size and trait heritability. Given the number of possible genes controlling maturity of fruit and the number of F_3 families analyzed (116; moderate), it is predictable that only QTL with comparatively large effects would be identified. These findings parallel those observed in tomato where two major early flowering-associated QTL explained the 3-week differences in flowering time and, hence, early FM (Lindhout et al. 1994).

Breeding strategies for enhanced QβC and early fruit maturity

No correlation between fruit mesocarp QβC and FM was detected in among the F_3 families. However, QTL for FM and QβC in endocarp, which have opposite effects [QTL (FM.6.1 LOD = 14.3, $R^2 = 35\%$) and QTL (β-carE.6.1, LOD = 7.27, $R^2 = 4.0\%$)] are located near each other in this population (LG VI; Fig. 1). Since the phenotypic variation explained by β-carE.6.1 is relatively low and endocarp tissue (i.e., seed cavity) is not consumed, selection for FM alleles may not result in significant, nutritionally important decreases of QβC in fruits. In fact, recombinant genotypes that carry a positive allele for QβC without negative associations with late maturity might be identifiable among novel, nearly isogenic lines differing in FM and QβC.

Phenotypic selection alone may not be effective in identifying an early US Western Shipping melon that produces fruits with high QβC (>25 μg g^{-1} in fruit mesocarp). The selection for FM presents additional complications since

its inheritance is complex (i.e., many genes with relatively small effects). This, and the fact that orange flesh color is under the control of two major epistatic genes and FM can be dramatically affected by the environment, necessitates the replicated evaluation of large segregating cross-progeny families (e.g., F_{3-4} and BC_{2-3}) over several locations.

Gain from the selection for FM and QβC might be enhanced by MAS if QTL-trait associations were definitive and consistent over generations of selection. Although, some of the QTL detected did not explain substantial amounts of the phenotypic variation ($R^2 \leq 20\%$; Table 4), appreciable ΔG has been achieved for the selection of complex traits in cucumber when MAS employed QTL that explained relatively small portions of the phenotypic variation [$R^2 = 5$–20%; (Fazio et al. 2003; Fan et al. 2006)]. One strategy for incorporating early FM and QβC into a population is by employing MAS, using QTL β-carM/E.9.1 in the first true-leaf stage, to identify plants that would potentially bear fruit with relatively high QβC (i.e., orange flesh). This strategy would permit the phenotypic field evaluation of progeny to identify early maturating individuals that develop fruit with the most intense hues of orange color. Fruit of such plants would undergo biochemical analysis to determine plants (families) that possess the highest QβC. This approach would allow the preliminary "in-field" assessment of a relatively large, early FM and QβC genotyped plant population, and the subsequent retention of selected plants by meristem propagation and/or the identification of the best performing advanced families.

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